microtubules, which are marked with specific posttranslational modifications (PTMs). We hypothesize that PTMs of tubulin directly influence the interaction of kinesin-1 with microtubules. In our previous work, we concluded that kinesin-1 could not identify the enzymatic addition or removal of acetylation to K40 of α-tubulin since this modification did not directly affect its in vitro motility properties. In vivo, PTMs are rarely found in isolation of one another. In fact, there is an extensive overlap between acetylated and detyrosinated microtubules in vivo. Therefore we set out to directly test the influence of detyrosination on kinesin-1 motility. To obtain populations of tyrosinated and detyrosinated tubulin, we purified 99.5% tyrosinated tubulin from HeLa cells which was then used to generate detyrosinated microtubules by in vitro treatment with carboxypeptidase A. In order to examine the effect of microtubule detyrosination on kinesin -based transport, we characterized the single molecule motility properties of fluorescently labeled kinesin-1 on tyrosinated and detyrosinated microtubules using Total Internal Reflection Fluorescence (TIRF) microscopy. We observed that kinesin-1 shows enhanced binding to detyrosinated microtubules resulting in a marked difference in motility for the modified microtubules when compared with tyrosinated microtubules. Our results suggest that the exposure of a negatively charged glutamate residue upon detyrosination increases the electrostatic binding between the kinesin motor and the microtubule. We conclude that the kinesin-1 shows enhanced binding to detyrosinated microtubules and this may be an important molecular event underpinning the preferential transport by kinesin-1 observed in vivo.

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Regulation of Axonal Transport by Kinesin Phosphorylation at S176

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The means by which the neuron regulates axonal transport of cargo remains an open field for study. Recent experiments examining Huntington's disease (HD) have revealed a molecular basis underlying fast axonal transport deficits characteristic of HD. This mechanism involves activation of JNK3 and JNK3-mediated phosphorylation of the kinesin-1 heavy chain (KHC) at serine 176. We have generated a phosphomimetic KHC construct (S176D) to characterize the effects phosphorylation has on kinesin's ability to translocate along microtubules. Using total internal reflection florescence (TIRF) microscopy, we have measured a dramatic reduction in the processive run length of S176D, as compared to the unphosphorylatable variant S176A. While this observation reveals a mechanism by which neurons control anterograde trafficking, it also suggests a mechanism for tuning the bidirectional transport of vesicles by kinesin and dynein.

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Detachment Kinetics of Single Kinesin and Dynein

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Intra-cellular transport via the microtubule motors kinesin and dynein plays an important role in maintaining cell structure and function. Often, multiple kinesin or dynein motors move the same cargo. Their collective function depends critically on the single motors' detachment kinetics under load. Single Kinesin's and Dynein's super-force off rates have been measured using an optical-trap based method. We rapidly increased the force on a moving bead and measured the time to detachment. From such events, detachment time distributions for specific super-force values have been measured. In contrast to a possible constant off-rate kinesin has an off-rate increasing with force. At low loads, dynein is sensitive to load; detaching easily but at higher load it exhibited a catch-bond type behavior, with off rate decreasing with load. The super-force experiments also allowed us to determine the probability of backward stepping for the motors. Kinesin and dynein can back-step under load, but this was relatively rare in both directions (<20%), and the typical backward travel distance was short.

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The Loop 5 Element Structurally and Kinetically Coordinates Dimers of the Human Kinesin-5 Eg5

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Eg5 is a homotetrameric kinesin-5 motor protein that generates outward force on the overlapping, antiparallel microtubules (MTs) of the mitotic spindle. Upon binding a MT, an Eg5 dimer releases one ADP molecule, undergoes a slow (~0.5 s-1) isomerization, and finally releases a second ADP, adopting a tightly MT-bound, nucleotide-free (APO) conformation. This conformation precedes ATP binding and stepping. Here, we use mutagenesis, steady-state and presteady-state kinetics, motility assays, and electron paramagnetic resonance (EPR) spectroscopy to examine Eg5 monomers and dimers as they bind MTs and initiate stepping. We demonstrate that a critical element of Eg5, loop 5 (L5), accelerates ADP release during the initial MT-binding event. Furthermore, our EPR data show that L5 mediates the slow isomerization by preventing Eg5 dimer heads from binding the MT until they release ADP. Finally, we find that Eg5 having a 7-residue deletion within L5 can still hydrolyze ATP and move along MTs, suggesting that L5 is not required to accelerate subsequent steps of the motor along the MT. Taken together, these properties of L5 completely explain the kinetic effects of L5-directed inhibition on Eg5 activity and may direct further interventions targeting Eg5 activity.

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Incorporation of Photochromic Molecule into the Functional Loop L5 of Kinesin for the Purpose of Photo-Regulation

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ATP driven molecular motor kinesin has several unique loops, which may determine the characteristic properties of kinesin. L5 is one of the unique loops locates in the vicinity of ATP binding site. We have previously demonstrated that the point mutations in the loop drastically affect ATPase activity. Moreover, it seems that the plus-end directed kinesins have longer L5 loop than that of minus-end directed kinesins. Therefore, the loop may determine the directionality of kinesin. Rice plant kinesin K16 has much shorter L5 than that of conventional kinesin. We have prepared the K16 mutant Q101C and H102C that have a single reactive cysteine reside in the L5. SH group reactive photochromic molecule composed of azobenzene derivative, N- (4-phenylazophenyl) maleimide (PAM) was incorporated into the cysteine residue in L5 to induce conformational change of L5 by ultraviolet (UV) and visible (VIS) light irradiation. The kinesin modified by PAM slightly altered the ATPase activity by UV and VIS light irradiation reversibly. We have also focused on the mitotic kinesin Eg5 that has an unusually elongated L5. It is known that the L5 of Eg5 has an important role to stabilize sterically its specific inhibitors (monastrol, STLC) that bind to the pocket near the ATPase site. We designed Eg5 mutants, E116C, E118C, D130C, A133C and Y125C to introduce photochromic molecule into the L5 of Eg5. The Effect of photoisomerization of azobenzene derivatives incorporated into L5 upon UV-VIS light irradiation for the inhibition of ATPase activity by monastrol or STLC was studied.

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Characterization of the Microtubules Modified with Photochromic Molecules

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Previously we have introduced photochromic molecules into the functional region of kinesin and succeeded to regulate the ATPase activity of kinesin reversibly by light irradiation. However, the regulation efficiency of the ATPase activity was not significant. It is well known that the contraction of skeletal muscle is regulated by calcium binding to the rail protein actin filament. The regulating system achieves high efficient switching. Therefore, it is expected that introducing artificial switching system into the rail protein microtubules enables high efficient regulation of kinesin motor activity. In this study, we tried to introduce photochromic molecules into the microtubules to regulate kinesin movement. First we modified the microtubules composed of wild type tubulin and measured gliding speed for kinesin using invitro motility assay under UV or VIS light irradiation, respectively. Gliding speeds of the microtubules modified with 4-phenylazophenyl maleimide (PAM) or 3,3-dimethyl-6'-nitro −1-[2- [3-(3- pyrroline −2,5-dion) −1-yl] propanoyloxy] ethylindoline −2-spiro-2' (2H)- chromene (MASP) were almost identical to intact microtubules and also were not influenced by UV or VIS light irradiation. This result suggested that that these photochromic molecules incorporated into intrinsic reactive cysteine residues on the surface of microtubules did not affect kinesin motor activity. Subsequently, we prepared the 4 types of α-tubulin mutants (V406C, V410C, M414C, G417C), which have additional cysteine residues in the kinesin binding region. Microtubule concentration dependent kinesin ATPase activity for the mutant microtubules, V406C, V410C and M414 showed almost similar values to that of WT microtubules. On the other hand, ATPase activity for G417C mutant microtubule drastically decreased. The